3

ANTISENSE MODULATION OF ANGIOTENSIN II AT1 RECEPTOR EXPRESSION IN ORGANOTYPIC CULTURES OF RAT BRAINSTEM. G.E. Gonye*, P. Hartig*, J.S. Schwaber#. *Central Nervous System Diseases Research, Dupont Merck Pharmaceutical Company and #Neurocomputation Group, Central Research Department, DuPont Co. Experimental Station, Wilmington, DE 19880.

The nucleus of the solitary tract (NTS) contains cells expressing receptors for a large number of neuropeptides (greater than twenty). This region of the brainstem plays a key role in the control of all autonomic functions, in particular the baroreflex-mediated regulation of blood pressure. Angiotensin II (ANG) causes well documented effects on blood pressure regulation, and in the brainstem, its AT1 receptors are expressed almost exclusively in the NTS. We have chosen the ANG/AT1 system as a test system to validate a widely applicable approach to study neuropeptide-driven modulation of function at the cellular level. We have combined organotypic culture and antisense knockdown to facilitate receptor level perturbation and subsequent whole cell patch analysis. Antisense oligonucleotide knockdown of receptor expression mimics receptor antagonists yet can circumvent the need for pharmaceutical reagents when none are available. Further, the required level of specificity demanded in receptor/ligand systems consisting of many closely related subtypes can be achieved. Organotypic brain culture has proven to be a functional intermediate between intact tissue and clonal cell lines preserving most of the organization and function of in vivo experiments. We have developed a culture system based on the "membrane interface" method of Stoppini et al. (J Neuro. Meth. 37: 173-182, 1991) using perinatal rat brainstem. AT1 expression in the rat brainstem was visualized by in situ hybridization from postnatal day 0 (P0) through P20 allowing us to define the most appropriate region and age from which to initiate cultures. P10 animals were chosen due to the sufficient level and extent of expression and the difficulty of generating viable organotypic cultures from older animals. The integrity of the culture system was demonstrated by Nissl stain, tyrosine hydroxylase expression, and patch clamp analysis. After 5 days in vitro (5DIV) major dorsal structures of the caudal medulla (NTS, X and XII nuclei motor cells, area postrema, and solitary tract) were easily identified. Expression of tyrosine hydroxylase mimicked in vivo expression temporally and spatially. Spontaneous activity of functionally identified motor cells (nucleus X cells backlabelled from the vagus nerve) showed the characteristic EPSP's and clustered spiking of intact synaptic function. The ability to deliver phosphothioate-based oligonucleotides (S-oligos) to cells within the constraints of the culture system was demonstrated by imaging the appearance of biotinylated S-oligos in the tissue from the media. Penetration was rapid (<4 h plateau) and through the entire thickness of the tissue. The vast majority of cells demonstrated varying degrees of oligonucleotide uptake, however a small subset appeared unlabelled. No overt toxicity was seen (5 uM S-oligo). AT1 mRNA expression was demonstrated after 5DIV by RT-PCR. AT1 protein levels are being determined by in situ receptor autoradiography and membrane binding assays. A kinetic analysis of expression as a function of culture time in the presence or absence of a panel of S-oligos is underway. We hope to demonstrate that the effects of knockdown on electrophysiology of ANG-responsive neurons will mirror effects caused by Losartan, an AT1-specific antagonist. Once validated, this approach should prove widely applicable to ligand/receptor systems where no pharmaceutical reagents are available, and, ultimately, if the sole reagent available is the cDNA sequence.

4

CLONING AND EXPRESSION OF HUMAN BRAIN BOMBESIN RECEPTORS. M.D. Hall, N. Suman-Chauhan, R. Pinnock, G.N. Woodruff, Parke-Davis Neuroscience Research Centre, Hills Road, Cambridge, UK.

The mammalian bombesin-like peptides, neuromedin B (NMB) and gastrin releasing peptide (GRP) are neuropeptides with a wide variety of biological activities. DNA encoding both bombesin receptor subtypes were obtained by PCR amplification from human brain cDNA. The NMB-receptor was cloned from caudate nucleus and the GRP-receptor from cortex. Sequence analysis confirmed that the cDNAs were identical to published sequences obtained from human small cell lung carcinoma cell lines. Stable CHO-K1 cell lines were obtained expressing each receptor subtype. The actions of NMB, neuromedin C and the GRP-receptor antagonist [D-Phe^{6}]-BN(6-13)-ethylamide were investigated on each receptor expressing cell line. Expression of receptors was also examined in the Xenopus oocyte expression system.